

CLAIMS

We claim:

1 1. A composition comprising a polymerizing agent including at least one molecular and/or
2 atomic tag located at or near, associated with or covalently bonded to a site on the polymerizing
3 agent, where a detectable property of the tag undergoes a change before, during and/or after
4 monomer incorporation.

1 2. The composition of claim 1, wherein the detectable property has a first value when the
2 polymerizing agent is in a first state and a second value when the polymerase is in a second state,
3 and where the polymerizing agent changes from the first state to the second state and back again
4 during each monomer incorporation.

1 3. The composition of claim 2, wherein the polymerizing agent is a polymerase or reverse
2 transcriptase.

1 4. The composition of claim 3, wherein the polymerase is selected from the group consisting
2 of *Taq* DNA polymerase I, T7 DNA polymerase, Sequenase, and the Klenow fragment from *E. coli*
3 DNA polymerase I.

1 5. The composition of claim 3, wherein the reverse transcriptase comprises HIV-1 reverse
2 transcriptase.

1 6. The composition of claim 3, wherein the polymerase comprises *Taq* DNA polymerase I
2 having a tag attached at a site selected from the group consisting of 513-518, 643, 647, 649 and 653-
3 661 and mixtures or combinations thereof of the *Taq* polymerase, where the tag comprises a
4 fluorescent molecule.

1 7. A composition comprising a polymerase or reverse transcriptase including at least one
2 molecular and/or atomic tag located at or near, associated with or covalently bonded to a site on the
3 polymerase, where a detectable property has a first value when the polymerase is in a first state and
4 a second value when the polymerase is in a second state during monomer incorporation, and where
5 the polymerizing agent changes from the first state to the second state and back again during each

6 monomer incorporation.

1 8. The composition of claim 7, wherein the polymerase is selected from the group consisting
2 of *Taq* DNA polymerase I, T7 DNA polymerase, Sequenase, and the Klenow fragment from *E. coli*
3 DNA polymerase I.

1 9. The composition of claim 7, wherein the reverse transcriptase comprises HIV-1 reverse
2 transcriptase.

1 10. A composition comprising a polymerizing agent including a molecular and/or atomic tag
2 associated with or covalently bonded to a site on the polymerase and a monomer including a
3 molecular and/or atomic tag, where at least one of the tags has a detectable property that undergoes
4 a change before, during and/or after monomer incorporation due to an interaction between the
5 polymerizing agent tag and the monomer tag.

1 11. The composition of claim 10, wherein the change in the detectable property results from a
2 change in the conformation of the polymerase from a first conformational state to a second
3 conformational state and back again during each monomer incorporation.

1 12. The composition of claim 10, wherein the detectable property has a first detection propensity
2 when the polymerase is in the first conformational state and a second detection propensity when the
3 polymerase is in the a second conformational state.

1 13. The composition of claim 12, wherein the polymerizing agent is a polymerase or reverse
2 transcriptase.

1 14. The composition of claim 13, wherein the polymerase is selected from the group consisting
2 of *Taq* DNA polymerase I, T7 DNA polymerase, Sequenase, and the Klenow fragment from *E. coli*
3 DNA polymerase I.

1 15. The composition of claim 13, wherein the reverse transcriptase comprises HIV-1 reverse
2 transcriptase.

1 16. The composition of claim 12, wherein the monomer comprise a dNTP and the tag is
2 covalently bonded to the β or γ phosphate group.

1 17. The composition of claim 10, wherein the tag comprises a fluorescent tag and the detectable
2 property comprises an intensity and/or frequency of emitted light.

1 18. The composition of claim 16, wherein the detectable property is substantially active when
2 the polymerase is in the first conformational state and substantially inactive when the polymerase
3 is in the second conformational state or substantially inactive when the polymerase is in the first
4 conformational state and substantially active when the polymerase is in the second conformational
5 state.

1 19. The composition of claim 14, wherein the polymerase comprises *Taq* DNA polymerase I
2 having a tag attached at a site selected from the group consisting of 513-518, 643, 647, 649 and 653-
3 661 and mixtures or combinations thereof of the *Taq* polymerase, where the tag comprises a
4 fluorescent molecule.

1 20. A composition comprising a polymerase or reverse transcriptase including a pair of tags
2 located at or near, associated with or covalently bonded to a site of the polymerase, where a
3 detectable property of at least one of the tags undergoes a change before, during and/or after
4 monomer incorporation.

1 21. The composition of claim 20, wherein the detectable property has a first value when the
2 polymerase is in a first state and a second value when the polymerase is in a second state, and where
3 the polymerizing agent changes from the first state to the second state and back again during each
4 monomer incorporation.

1 22. The composition of claim 21, wherein the polymerase is selected from the group consisting
2 of *Taq* DNA polymerase I, T7 DNA polymerase, Sequenase, and the Klenow fragment from *E. coli*
3 DNA polymerase I.

1 23. The composition of claim 21, wherein the reverse transcriptase comprises HIV-1 reverse
2 transcriptase.

1 24. The composition of claim 22, wherein the polymerase comprises *Taq* DNA polymerase I
2 having a tag attached at a site selected from the group consisting of 513-518, 643, 647, 649 and
3 653-661 and mixtures or combinations thereof of the *Taq* polymerase, where the tag comprises a
4 fluorescent molecule.

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25. A single molecule sequencing apparatus comprising a substrate having a first chamber in
which at least one tagged polymerase is confined therein and a second chamber including tagged
dNTPs and a channel interconnecting the chambers, where a detectable property of at least one tag
undergoes a detectable change during a monomer incorporation cycle.

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26. The apparatus of claims 24, further comprising a plurality of monomer chambers, one for
each tagged dNTP.

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27. A mutant *Taq* polymerase comprising native *Taq* polymerase with a cysteine residue
replacement at a site selected from the group consisting of 513-518, 643, 647, 649 and 653-661 and
mixtures or combinations thereof.

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28. The polymerase of claim 27, wherein the cysteine residue includes a tag covalently bonded
thereto through the SH group.

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29. A system for retrieving stored information comprising:
a unknown nucleotide sequence representing a data stream;
a single-molecule sequencer including a polymerase having a tag associated therewith and
monomers for the polymerase, each monomer having a tag associated therewith;
an excitation source adapted to excite the at least one of the tags; and
a detector adapted to detect a response from at least one of the tag,
where the response changes during polymerization of a complementary sequence and the
changes in response represent a content of the data stream.

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29. A system for determining sequence information from a single molecule comprising:
a unknown nucleotide sequence;
a single-molecule sequencer comprising a polymerase having a tag associated therewith and
monomers for the polymerase, each monomer having a tag associated therewith;
an excitation source adapted to excite at least one of the tags; and
a detector adapted to detect a response from at least one of the tags,
where the response changes during polymerization of a complementary sequence and the
changes in the response represent the identity of each nucleotide in the unknown sequence.

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30. A method for sequencing a molecular sequence comprising:
supplying an unknown sequence of nucleotides or nucleotide analogs to a single-molecule
sequencer comprising a polymerase having a fluorescent donor covalently attached thereto and
monomers for the polymerase, each monomer having a unique fluorescent acceptor covalently
bonded thereto;
exciting the fluorescent donor with a light from an excitation light source;
detecting emitted fluorescent light from the acceptor during a monomer incorporation cycle
via a fluorescent light detector, where an intensity and/or frequency of the emitted light for the
acceptors changes during each monomer incorporation cycle; and
converting the changes into an identity of each nucleotide or nucleotide analog in the
unknown sequence.

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31. A method of sequencing an individual nucleic acid molecule or numerous individual
molecules in parallel including the steps of:
immobilizing a member of the replication complex comprising a polymerase including a tag
attached thereto, a primer or a template sufficiently spaced apart to allow resolution detection of
each complex on a solid support;
incubating the replication complex with cooperatively-tagged nucleotides, each nucleotide
including a unique tag at its gamma-phosphate, where each nucleotide can be individually detected;
detecting each nucleotide incorporated by the polymerase as the polymerase transitions
between its open and closed form, which causes a change in a detectable property of at least one of
the tags or as the pyrophosphate group is released by the polymerase; and
relating the changes in the detectable property to the sequence of nucleotides in an unknown

12 nucleic acid sequence.

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1 32. A γ -phosphate modified nucleoside comprising γ -phosphate modified dATP, dCTP, dGTP
2 and dTTP.

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1 33. A primer sequence or portion thereof selected from the group consisting of Sequence 1
2 through 29.

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